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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The major accomplishment under this contract
Our major accomplishment under Contract N00014-72-C-0210 was the development and testing of a treatment model using mice to evaluate transplantation of liver and thymus cells from immunologically immature (fetal) donors as a source of hematopoietic stem cells. The following significant observations were made: (1) (cont over)

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Block 20 (continued):

a) Fetal liver cells can be used as a source of hematopoietic stem cells without the complication of acute graft-versus-host disease; (2)

b) Recovery from radiation injury was dependent on the dose of fetal liver cells transplanted; (3)

c) Transplanting very small numbers of fetal thymus cells, in addition to liver cells, had a significant and salutary effect on recovery from radiation injury in some strain combinations, but not in others; (4)

d) Transplantation of fetal liver cells from histoincompatible unrelated donors resulted in less delayed secondary disease mortality than did bone marrow transplants from histocompatible unrelated donors; (5)

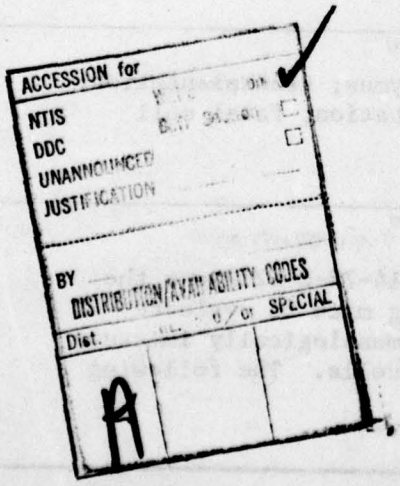
e) Cell yields from human embryonic tissue increased exponentially with age, and the liver:thymus ratio was 35.4:1; and (6)

f) Fetal liver (+ or −) thymus cells promoted recovery in lethally irradiated adult mice when given in a cell dose per kg body weight comparable to that from a human fetus at 14 weeks embryonation. ☆

g) The addition of fetal thymus tissue to fetal liver cells did not result in a significant increase in the quantity of hematopoietic stem cells transplanted.

h) There was no relationship between the age of the fetal liver-thymus donor and the incidence of delayed secondary disease.

i) Polyclonal activators of B lymphocytes, in some instances, resulted in a significant decrease in mortality from delayed secondary disease following allogeneic bone marrow transplantation.



OFFICE OF NAVAL RESEARCH

Contract N00014-72-C-0210

FINAL REPORT

GRAFT VERSUS HOST INHIBITION. XI. The Therapeutic Potential of Combined Fetal Liver and Thymus Cells as a "Universal" Hematopoietic Stem Cell Source for Transplantation.

by

Mortimer M. Bortin, M.D.

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18 January 1978

I. Introduction

A rapidly increasing number of military personnel and civilians are at risk of accidental exposure to potentially lethal radiation associated with an increase in the use of nuclear power sources. In addition, there is increasing concern regarding the risk of radiation fallout from nuclear tests or warfare. Exposure of mammals to large doses of radiation destroys their blood-forming systems and causes death because of irreparable damage to their hematopoietic stem cells. Since 1951, transplantation of bone marrow cells from normal donors has been used to reconstruct the blood-forming systems of animals following supralethal doses of radiation. However, between 1951 and 1968 all attempts to apply bone marrow transplantation in man were unsuccessful. In 1968, the principal investigator along with Dr. Fritz H. Bach and our colleagues performed the first successful human bone marrow transplant. The key to our success was that donor and recipient were identical at HLA, the major histocompatibility complex (MHC) in man. Shortly thereafter, others successfully transplanted patients using donors who were closely matched at the MHC. Almost all successful human bone marrow transplants have been accomplished when donors and recipients were MHC-compatible siblings. Unfortunately, most individuals do not have MHC-compatible siblings. It has been estimated that between 10,000 and 50,000 individuals in the general population would have to be screened to find a perfectly matched MHC-compatible donor. Even if an unrelated MHC-compatible individual were found, there is no assurance that he would consent to the pain and risks attendant to bone marrow donation.

All experiments performed under ONR Contract N00014-72-C-0210 were aimed at developing methods to treat radiation casualties for whom there was no MHC-compatible donor available. The main thrust of the work was to investigate the possibility that hematopoietic stem cells obtained following death of immunologically immature fetuses could be transplanted in place of bone marrow and serve as a "Universal" or "Type-0" donor.

II. Summary of Research Accomplished

The purpose of the research conducted under Contract N00014-72-C-0210 was to develop a feasible treatment plan which could be used to treat personnel exposed to supralethal doses of radiation (e.g., from nuclear accidents, from radiation fallout, etc.). The treatment of choice would be MHC-compatible bone marrow transplantation; however, finding MHC-compatible donors of bone marrow for large numbers of radiation casualties would appear to be an insuperable problem. On the basis of the research conducted under this Contract, it is

apparent that transplantation of hematopoietic stem cells from fetal donors is a viable alternative for the treatment of radiation casualties when an MHC-compatible donor cannot be found. Summarized below are experiments conducted between 1972 and 1977 in an effort to define the factors which influenced success and failure of fetal tissue transplants for the treatment of radiation disease.

In the initial set of experiments we sought to determine if it might be possible to circumvent the transplantation problems posed by the small numbers of hematopoietic stem cells present in a single human fetus. Lethally irradiated adult CBA ($H\text{-}2^k$) mice were transplanted with small numbers of liver hematopoietic cells from strain A ($H\text{-}2^a$) fetal donors. Recovery from radiation injury was dependent upon the dose of liver cells administered. Giving very small numbers of fetal thymus cells, in addition to liver cells, had a highly significant and salutary effect on survival. The experimental results suggested that transplantation of liver cells from a single human fetus along with thymus cells might successfully reconstruct the hematopoietic system of an adult human radiation casualty.

Cell yield data were obtained on the number of liver and thymus cells obtainable at different gestational ages of human embryos and fetuses. Growth was exponential in both organs and the mean liver to thymus ratio was 35.4:1. This information provided the basis for later estimates of the quantity of liver hematopoietic cells that could be recovered from a single 14 week-old human fetus for transplantation.

A series of experiments was initiated using a mouse model to evaluate: (1) whether lethally irradiated mice would survive when transplanted with fetal liver cells from immunologically immature MHC-incompatible donors by using a cell dose per kilogram body weight comparable to that which would be available clinically from a single human fetus at fourteen weeks of embryonation; (2) whether the addition of a small number of fetal thymus cells to the fetal liver inoculum, would influence hematopoietic restoration and long-term survival; and (3) whether transplants of cells from MHC-incompatible fetal donors would promote higher long-term survival as compared to bone marrow cells from allogeneic MHC-compatible adult donors. CBA mice were exposed to a supralethal dose of total body irradiation and given transplants of graded doses of bone marrow, fetal liver or fetal liver plus fetal thymus cells obtained from MHC-compatible C58 or MHC-incompatible strain A donors. Survival at 20 days was used to evaluate the ability of the transplant to restore hematopoiesis following

acute radiation injury. In the higher dose ranges, the fetal cells were as effective as adult bone marrow in both the MHC-compatible and incompatible strain combinations. Survival at 100 days was used to evaluate the severity of delayed secondary disease following transplantation. In the higher dose ranges, cells from fetal donors promoted higher long-term survival rates than did comparable dosages of bone marrow cells in both the matched and mismatched combinations. In some experimental groups, the addition of fetal thymus cells to fetal liver cells resulted in higher short-term and long-term survival rates than did fetal liver alone, but this was inconsistent and generally fell short of statistical significance. The most important finding was that cells from MHC-incompatible, unrelated fetal donors (using a cell dose per kilogram body weight comparable to the number of fetal liver and thymus cells which would be obtainable from one human fetus at 14 weeks of embryonation) promoted higher long-term survival rates than did bone marrow transplants from matched, unrelated donors.

Information obtained from experiments in the mouse model were applied to fetal liver cell transplantation in dogs in a collaborative study with Edward C. Saltzstein, M.D.,Chairman of the Department of Surgery at our institution. A lethally irradiated female dog was transplanted with MHC-incompatible fetal liver and thymus cells. Prompt recovery from the radiation injury occurred, and there was no evidence of acute GVH disease or delayed secondary disease. To our knowledge, this was the first successful production of a long-lived canine allogeneic radiation chimera in which fetal donors were used as the source of hematopoietic stem cells.

The next set of experiments were carried out in collaboration with Walter Fried, M.D., Professor, Department of Medicine, Abraham Lincoln School of Medicine, Chicago. The purpose of this study was to determine the effect of fetal thymus cells on hematopoiesis in syngeneic radiation chimeras transplanted with fetal liver cells using the Till and McCulloch spleen colony forming unit (CFU-S) assay. Stem cell repopulation in the femoral bone marrow of lethally irradiated mice was not significantly different in mice given transplants of fetal liver with or without fetal thymus.

In the first phase of the next series of experiments we determined the dose of liver cells from perinatal donors at different stages of development which would provide 50% long term survival in three strain combinations. The strain combinations tested were: A ($H\text{-}2^a$) → CBA ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$) → CBA, and CBA → CBA. Donor tissue was taken at 14, 16, 18 and 26 days post-conception. Three to five cell doses of

fetal liver cells were tested for each donor age. The appropriate dose of fetal liver cells was then selected for phase two experiments. In the second phase we sought to determine whether the addition of fetal thymus cells to fetal liver cells at various ages post-conception had a beneficial effect on hematopoiesis and long-survival following exposure to high doses of total body irradiation. Fetal liver cells were inoculated alone or with thymus cells from donors of the same age in a 30:1 (Liver:thymus) ratio. Within the contraints of the experimental design employed, there was no statistically significant relationship between the age of fetal liver/fetal thymus donor and the incidence of lethal secondary disease.

In the final set of experiments we tested the hypothesis that survival of allogeneic radiation chimeras could be improved by increasing and/or accelerating B cell function post-transplant. Two donor-recipient combinations known to produce delayed secondary disease mortality were used. Three polyclonal B cell activators (PBAs) were tested; dextran sulfate (DxS), purified protein derivitive (PPD) from tuberculin, and bacterial lipopolysaccharide (LPS). The PBAs were used individually and in various combinations in the allogeneic radiation chimeras. Fetal thymus cells from a "third party" were transplanted in addition to allogeneic bone marrow and lymph node cells in an effort to accelerate restoration of the T cell immune system. The data indicated that the PBAs in the combinations of DxS plus LPS or DxS plus PPD with or without fetal thymus cells were ineffective at preventing secondary disease mortality in the strain combinations tested. The most important finding in this set of experiments was that when DxS was given alone over a long period of time there was a significant ($P < 0.002$) decrease in secondary disease mortality for one of the two allogeneic strain combinations tested.

III. Index of All Technical Reports

No technical reports have been submitted.

IV. Bibliography of Publications

Saltzstein, E.C., Rimm, A.A. and Bortin, M.M.: Graft versus Host Inhibition. VI. Reduced Secondary Disease Mortality in Murine Allogeneic Radiation Chimeras with Low Dose Fetal Liver and Thymus Cells in Combination. Exp. Hematol. 1:291, 1973.

Bortin, M.M., Rimm, A.A. and Saltzstein, E.C.: Potentiating Effect of Fetal Thymus on Fetal Liver Cells for the Promotion of Recovery from the Radiation Injury in Murine Allogeneic Radiation Chimeras, or A Little Bit of Thymus Goes a Long, Long Way. In: Birth Defects: Original Articles Series,

Vol. II, Immunodeficiency in Man and Animals (D. Bergsma, ed.), Sinauer Associates, Inc., Sunderland, MA, 1975, pp. 544-546.

Bortin, M.M., Rimm, A.A., Rose, W.C., Truitt, R.L. and Saltzstein, E.C.: Transplantation of Hematopoietic and Lymphoid Cells in Mice: H-2 Matched Unrelated Adult Donors Compared with H-2 Mismatched Fetal Donors. Transplantation 21:331-336, 1976.

V. List of Major Accomplishments

Our major accomplishment under Contract N00014-72-C-0210 was the development and testing of a treatment model using mice to evaluate transplantation of liver and thymus cells from immunologically immature (fetal) donors as a source of hematopoietic stem cells. The model was designed so that it would be applicable for the treatment of large numbers of radiation casualties. Our research results were presented at several national and international meetings of scientists concerned with radiation injury and the value of hematopoietic cell transplantation. In the pursuance of our research objectives we have made the following significant observations:

a) Liver cells from immunologically immature mouse embryos (fetuses) can be used as a source of hematopoietic stem cells for transplantation without the complication of acute graft-versus-host disease;

b) Recovery from radiation injury was dependant on the dose of fetal liver cells transplanted;

c) Transplanting very small numbers of fetal thymus cells, in addition to liver cells, had a highly significant and salutary effect on recovery from radiation injury in some strain combinations, but not in others;

d) Transplantation of fetal liver cells from MHC-incompatible unrelated donors promoted higher long-term survival rates (i.e., less delayed secondary disease) than did bone marrow transplants from MHC-compatible unrelated donors;

e) Cell yields from human embryonic tissue increased exponentially with age, and the liver:thymus ratio was 35.4:1.

f) Fetal liver ± thymus cells promoted recovery in adult mice exposed to supralethal doses of radiation when given in a cell dose per kilogram body weight comparable to the number of fetal liver and thymus cells which would be obtainable from one human fetus at 14 weeks of embryonation;

g) The addition of fetal thymus tissue to fetal liver cells did not result in a significant increase in the quantity of hematopoietic stem cells transplanted;

h) There was no significant relationship between the age of fetal liver/fetal thymus donors and the incidence of delayed secondary disease;

i) Transplantation of liver and thymus cells from MHC-incompatible fetal donors to a lethally irradiated adult dog reconstructed its hematopoietic system and resulted in the first long-lived allogeneic, canine radiation chimera produced with fetal cells; and

j) Polyclonal activators of B-lymphocytes, in some instances, resulted in a significant decrease in mortality from delayed secondary disease following allogeneic bone marrow transplantation.